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US 04/03878

P2 1155841

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

October 27, 2006

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY OF THE BELOW IDENTIFIED INTERNATIONAL APPLICATION AS ORIGINALLY FILED AND ANY CORRECTIONS THERETO FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE ACTING AS A RECEIVING OFFICE UNDER THE PATENT COOPERATION TREATY.

APPLICATION NUMBER: *PCT/US03/26356*
FILING DATE: *August 22, 2003*

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office

H. L. Jackson

H. L. JACKSON
Certifying Officer

COPY

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No. PCT/US 03/26356

International Filing Date 22 AUG 2003 (22.08.03)

Name of receiving Office and "PCT International Application" PCT INTERNATIONAL APPLICATION RO/US

Applicant's or agent's file reference (if desired) (12 characters maximum) 8399-009-228

Box No. I TITLE OF INVENTION

ASSEMBLY OF CHITOSAN ONTO AN ELECTRODE SURFACE

Box No. II APPLICANT ☐ This person is also inventor

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

UNIVERSITY OF MARYLAND BIOTECHNOLOGY INSTITUTE
9600 Gudelsky Drive
Rockville, MD 20850
US

Telephone No.

Facsimile No.

Teleprinter No.

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

UNIVERSITY OF MARYLAND BALTIMORE COUNTY
1000 Hilltop Circle
Baltimore, MD 21250
US

This person is:
☒ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

CORUZZI, Laura A.

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, NY 10036
US

Telephone No. 212-790-9090

Facsimile No. 212-869-9471

Teleprinter No.

Agent's registration No. with the Office

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

YI, Hyunmin
3907 Lakehouse Rd., Apt. #13
Beltsville, MD 20705
US

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: KR

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

RUBLOFF, Gary W.
13720 Springdale Drive
Clarksville, MD 21029
US

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BENTLEY, William E.
9513 Thornhill Road
Silver Spring, MD 20901
US

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

GHODSSI, Reza
5805 Edson Lane, #203
Rockville, MD 20852
US

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: IR

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

[X] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PAYNE, Gregory F.
P.O. Box 1304
Hunt Valley, MD 21030
US

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATION OF STATES *Mark the applicable check-boxes below; at least one must be marked.*

The following designations are hereby made under Rule 4.9(a):

Regional Patent

☒ **AP** **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZM Zambia, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

☒ **EA** **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP** **European Patent:** AT Austria, BE Belgium, BG Bulgaria, CH & LI Switzerland and Liechtenstein, CY Cyprus, CZ Czech Republic, DE Germany, DK Denmark, EE Estonia, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, HU Hungary, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, RO Romania, SE Sweden, SI Slovenia, SK Slovakia, TR Turkey, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA** **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GQ Equatorial Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AE United Arab Emirates .....
☒ AG Antigua and Barbuda
☒ AL Albania .....
☒ AM Armenia .....
☒ AT Austria .....
☒ AU Australia .....
☒ AZ Azerbaijan .....
☒ BA Bosnia and Herzegovina .....
☒ BB Barbados
☒ BG Bulgaria .....
☒ BR Brazil .....
☒ BY Belarus .....
☒ BZ Belize .....
☒ CA Canada
☒ CH & LI Switzerland and Liechtenstein
☒ CN China .....
☒ CO Colombia .....
☒ CR Costa Rica .....
☒ CU Cuba .....
☒ CZ Czech Republic .....
☒ DE Germany .....
☒ DK Denmark .....
☒ DM Dominica
☒ DZ Algeria .....
☒ EC Ecuador .....
☒ EE Estonia .....
☒ ES Spain .....
☒ FI Finland .....
☒ GB United Kingdom
☒ GD Grenada
☒ GE Georgia .....
☒ GH Ghana .....
☒ GM Gambia

☒ HR Croatia .....
☒ HU Hungary .....
☒ ID Indonesia
☒ IL Israel .....
☒ IN India .....
☒ IS Iceland
☒ JP Japan .....
☒ KE Kenya .....
☒ KG Kyrgyzstan
☒ KP Democratic People's Republic of Korea .....
☒ KR Republic of Korea .....
☒ KZ Kazakhstan .....
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho .....
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MA Morocco .....
☒ MD Republic of Moldova .....
☒ MG Madagascar .....
☒ MK The former Yugoslav Republic of Macedonia .....
☒ MN Mongolia
☒ MW Malawi .....
☒ MX Mexico .....
☒ MZ Mozambique .....
☒ NI Nicaragua
☒ NO Norway
☒ NZ New Zealand .....

☒ OM Oman
☒ PG Papua New Guinea
☒ PH Philippines .....
☒ PL Poland .....
☒ PT Portugal .....
☒ RO Romania
☒ RU Russian Federation .....
.....
☒ SC Seychelles
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SK Slovakia .....
☒ SL Sierra Leone .....
☒ SY Syrian Arab Republic
☒ TJ Tajikistan .....
☒ TM Turkmenistan .....
☒ TN Tunisia
☒ TR Turkey .....
☒ TT Trinidad and Tobago .....
.....
☒ TZ United Republic of Tanzania
☒ UA Ukraine .....
☒ UG Uganda .....
☒ US United States of America .....
.....
☒ UZ Uzbekistan .....
☒ VC Saint Vincent and the Grenadines
☒ VN Viet Nam .....
☒ YU Serbia and Montenegro .....
☒ ZA South Africa .....
☒ ZM Zambia
☒ ZW Zimbabwe .....

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ ..... ☐ ..... ☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Sheet No. .....

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

1. If, in any of the Boxes, except Boxes Nos. VIII(i) to (v) for which a special continuation box is provided, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No...." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) if more than two persons are to be indicated as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

(vi) if, in Box No. VI, there are more than five earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

CONTINUATION OF BOX NO. IV

TERZIAN, Berj A.; WEILD, III, David; REIN, Barry D.; MORRIS, Francis E.; STERN, Gidon D.; LAUTER, Jr., John J.; POISSANT, Brian M.; RADDING, Rory J.; CORUZZI, Laura A.; SHANNON, Philip T.; GOODELL, Donald J.; FRIEBEL, Thomas E.; BALDWIN, Geraldine F.; BALANCIA, Victor N.; ABRAMS, Samuel B.; ANTLER, Adriane M.; ROWAN, Thomas G.; MARKEY, James G.; KOHLER, Thomas D.; STIMPSON, Scott D.; WILLIAMS, Gary S.; GISOLFI, Ann L.; TALCOTT, Kelly D.; CERRITO, Francis D.; INSOGNA, Anthony M.; ROTHERY, Brian M.; SIFF, Brian D.; LYONS, Michael J.; GEORGE, Nikolaos C.; RABINOWITZ, Stephen S.; SHENTOV, Ognjan V.; STEIN, Kenneth L.; GRAY, Andrew J.; LEBOWITZ, Henry C.; MERKEN, Leo; BRIVANLOU, Margaret B.; OWENS, David R.; LANGER, Matthew E.; HOROWITZ, Karen G; TSANG, T. Christopher; BRETSCHER, Carl P.

Sheet No. .....

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (1) 23 AUG 2002 (23.08.02)	60/405,582	US		
item (2)				
item (3)				
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office)* identified above as:

☒ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see Supplemental Box

** Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):* ....

.....

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA /US .....

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII DECLARATIONS

The following **declarations** are contained in Boxes Nos. VIII (i) to (v) *(mark the applicable check-boxes below and indicate in the right column the number of each type of declaration)*: Number of declarations

☐ Box No. VIII (i)	Declaration as to the identity of the inventor	:
☐ Box No. VIII (ii)	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	:
☐ Box No. VIII (iii)	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	:
☐ Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the United States of America)	:
☐ Box No. VIII (v)	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	:

Box No. IX CHECK LIST; LANGUAGE OF FILING PCT/US03/26356

This international application contains:

(a) **in paper form**, the following number of sheets:

request (including declaration sheets)	:	7
description (excluding sequence listings and/or tables related thereto)	:	11
claims	:	2
abstract	:	1
drawings	:	6
Sub-total number of sheets	:	27
sequence listings	:	
tables related thereto	:	
(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c) below)		
Total number of sheets	:	27

(b) ☐ **only in computer readable form** (Section 801(a)(i))
- (i) ☐ sequence listings
- (ii) ☐ tables related thereto

(c) ☐ **also in computer readable form** (Section 801(a)(ii))
- (i) ☐ sequence listings
- (ii) ☐ tables related thereto

Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the
- ☐ sequence listings: .....
- ☐ tables related thereto: .....

(additional copies to be indicated under items 9(ii) and/or 10(ii), in right column)

This international application is **accompanied by** the following item(s) *(mark the applicable check-boxes below and indicate in right column the number of each item):*

		Number of items
1. ☒ fee calculation sheet	:	1
2. ☐ original separate power of attorney	:	
3. ☐ original general power of attorney	:	
4. ☒ copy of general power of attorney; reference number, if any:	:	1
5. ☐ statement explaining lack of signature	:	
6. ☐ priority document(s) identified in Box No. VI as item(s):	:	
7. ☐ translation of international application into *(language)*:	:	
8. ☐ separate indications concerning deposited microorganism or other biological material	:	
9. ☐ sequence listings in computer readable form *(indicate type and number of carriers)*		
(i) ☐ copy submitted for the purposes of international search under Rule 13*ter* only (and not as part of the international application)	:	
(ii) ☐ *(only where check-box (b)(i) or (c)(i) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Rule 13*ter*	:	
(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listings mentioned in left column	:	
10. ☐ tables in computer readable form related to sequence listings *(indicate type and number of carriers)*		
(i) ☐ copy submitted for the purposes of international search under Section 802(b-*quater*) only (and not as part of the international application)	:	
(ii) ☐ *(only where check-box (b)(ii) or (c)(ii) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-*quater*)	:	
(iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column	:	
11. ☒ other *(specify)*: Transmittal Letter	:	1

Figure of the drawings which should accompany the abstract: 1

Language of filing of the international application: English

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

David Weild, III

Agent for Applicant

For receiving Office use only

1. Date of actual receipt of the purported international application: (22.08.03) 1702 Rec'd PCT/PTO 22 AUG 2003

2. Drawings: ☐ received: ☐ not received:

3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:

4. Date of timely receipt of the required corrections under PCT Article 11(2):

5. International Searching Authority (if two or more are competent): ISA/US

6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT/US03/26356

PCT

FEE CALCULATION SHEET

Annex to the Request

For receiving Office use only

International Application No. PCT/US 03/26356

Date stamp of the receiving Office

Applicant's or agent's file reference 8399-009-228

Applicant

UNIVERSITY OF MARYLAND BIOTECHNOLOGY INSTITUTE et al.

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . 240.00 [T]

2. SEARCH FEE . . . . . 700.00 [S]

International search to be carried out by US

(If two or more International Searching Authorities are competent to carry out the international search, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

Where items (b) and/or (c) of Box No. IX apply, enter **Sub-total number of sheets**
Where items (b) and (c) of Box No. IX do not apply, enter **Total number of sheets** } 27

[b1] first 30 sheets . . . . . 476.00 [b1]

[b2] _____ (number of sheets in excess of 30) x 12 (fee per sheet) = [b2]

[b3] additional component (only if sequence listings and/or tables related thereto are filed in computer readable form under Section 801(a)(i), or both in that form and on paper, under Section 801(a)(ii)):

400 x _____ (fee per sheet) = [b3]

Add amounts entered at b1, b2 and b3 and enter total at B . . . . 476.00 [B]

Designation Fees

The international application contains ALL designations.

5 (number of designation fees payable *(maximum 5)*) x 104 (amount of designation fee) = 520.00 [D]

Add amounts entered at B and D and enter total at I . . . . . 996.00 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* 15 x 1 . . . 15.00 [P]

5. TOTAL FEES PAYABLE . . . . . 1,951.00 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

☒ authorization to charge deposit account (see below) ☐ postal money order ☐ cash ☐ coupons

☐ cheque ☐ bank draft ☐ revenue stamps ☐ other *(specify):*

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(This mode of payment may not be available at all receiving Offices)

Receiving Office: RO/ US

Deposit Account No.: 16-1150

Date: 22 AUGUST 2003

Name: David Weild, III

Signature: [signature]

☒ Authorization to charge the total fees indicated above.

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GENERAL POWER OF ATTORNEY

PATENT COOPERATION TREATY

Appointment of Agent or Common Representative

The undersigned applicant hereby appoints:

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/X/ as agent /__/ as common representative

to act on their behalf before the competent International Authorities in connection with any and all international applications filed with the RO/US, and to receive correspondence and payments on their behalf.

Dated: January 23, 1998.

UNIVERSITY OF MARYLAND BIOTECHNOLOGY INSTITUTE

Rita R. Colwell
President

ASSEMBLY OF CHITOSAN ONTO AN ELECTRODE SURFACE

This application claims the benefit of U.S. provisional application no. 60/405,582, filed August 23, 2002, the entirety of which is incorporated herein by reference. The U.S. government may have certain rights to this invention, pursuant to Grant No. BES-0114790, awarded by the National Science Foundation.

1. FIELD OF THE INVENTION

The invention relates to methods of depositing polysaccharide chitosan from a chitosan solution onto a substrate.

2. BACKGROUND OF THE INVENTION

The ability to create devices (*e.g.*, biosensors, microarrays, and microelectromechanical systems (MEMS)) requires facile methods to precisely control surfaces. A variety of patterning techniques can be used to produce desired structures, while various methods have been investigated to control surface chemistries. For instance, surface chemistries have been controlled by self-assembling monolayers using reactions between thiols and metal surfaces, or between alkyltrichlorosilanes and oxidized silicon. Bain, C. D., Whitesides, G. M. *Angew. Chem. Int. Ed. Engl.* 1989, *28*, 506-512; Whitesides, G. M., Laibinis, P. E. *Langm.* 1990, *6*, 87-96; Sagiv, J. *J. Am. Chem. Soc. 102*, 1980, 92-98; Brzoska, J. B., Azouz, I. B.; Rondelez, F. *Langm.* 1994, *10*, 4367-4373; Allara, D. L., Parikh, A. N., Rondelez, F. *Langm.* 1995, *11*, 2357-2360. An additional method to assemble macromolecules and particles is to exploit an applied voltage. Foo, G. M., Pandey, R. B. *Biomacromol.* 2000, *1*, 407-412. Applied voltages have been used to assemble colloidal particles, proteins, and cells onto electrode surfaces. Bohmer, M. *Langm.* 1996, *12*, 5747-5750; Strike, D. J., Rooij, N. F., de Koudelka-Hep, M. *Biosen. Bioelect.* 1995, *10*, 61-66; Cosnier, S. *Biosen. Bioelect.* 1999, *14*, 443-456; Kurzawa, C., Hengstenberg, A., Schuhmann, W. *Anal. Chem.* 2002, *74*, 355-361; Kurzawa, C., Hengstenberg, A., Schuhmann, W. *Anal. Chem.* 2002, *74*, 355-361; Brisson, V., Tilton, R. D. *Biotechnol. Bioeng.* 2002, *77*, 290-295.

Chitosan is an amine-rich polysaccharide derived by deacetylation of chitin. Chitin is the second most abundant polysaccharide in nature and is found in crustaceans, insects, and fungi. Chitosan is becoming an increasingly important biopolymer because it offers unique physicochemical properties. Hudson, S.M.; Smith, C. In *Biopolymers from Renewable Resources*, D.L. Kaplan (Ed.), Springer, Berlin, 1998, p. 96-118. Specifically, chitosan has primary amino groups that have pKa values of about 6.3. Rinaudo, M., Pavlov, G., Desbrieres, J. *Polymer* 1999, *40*, 7029-7032; Sorlier, P., Denuziere, A., Viton, C., Domard, A. *Biomacromolec.* 2001, *2*, 765-772. At pHs below the pKa, most of the amino groups are protonated making chitosan a water-soluble, cationic polyelectrolyte. Chitosan's water-solubility is unique as other β,(1→4)-linked polysaccharides (*e.g.*, cellulose and chitin) are insoluble. At pHs above the pKa, chitosan's amino groups are deprotonated, and this polymer becomes insoluble. Chitosan's pH-dependent solubility is attractive because it allows processing from aqueous solutions while a modest increase in pH to neutrality enables chitosan to be formed into various shapes (*e.g.*, beads, membranes, and films). Ligler, F.S., Lingerfelt, B.M., Price, R.P., Schoen, P.E. *Langm.* 2001, *17*, 5082-5084. An additional feature is that chitosan's amino groups confer nucleophilic properties to this polymer. Specifically, the deprotonated amino groups have an unshared electron pair that can undergo reaction with a variety of electrophiles. As a result, various chemistries can be exploited to crosslink chitosan and to graft substituents onto this polymer. Hirano, S., Ohe, Y., Ono, H. *Carbohydr. Res.* 1976, *47*, 315-320; Muzzarelli, R. A. A., Taniani, F., Emanuelli, M., Marioth, S. *Carbohydr Res.* 1982, *107*, 199-214; Yalpani, M., Hall, L.D. *Macromol.* 1984, *17*, 272-281; Roberts, G.A.F., Taylor, K.E *Die Makromolek. Chemie.* 1989, *190*, 951 – 960; Hsien, T.-Y., Rorrer, G.L. *Sep. Sci. Technol.* 1995, *30*, 2455-2475; Gruber, J.V., Rutar, V., Bandekar, J., Konish, P.N. *Macromolec.* 1995, *28*, 8865-8867; Xu, J., McCarthy, S.P., Gross, R.A., Kaplan D.L. *Macromolec.* 1996. *29*, 3436-3440; Knaul, J.Z., Hudson, S.M., Creber, K.A.M. *J. Polym. Sci.: B: Polym. Phys.* 1999, *37*, 1079-1094; Mi, F.-L., Kuan, C. Y., Shyu, S.-S., Lee, S. T., Chang, S. F. *Carbohydr. Polym.* 2000, *41*, 389-396; Mi, F.-L., Sung, H.-W., Shyu, S.-S. J. *Appl. Polym. Sci.* 2001, *81*, 1700-1711; Kurita, K., Ikeda, H., Yoshida, Y., Shimojoh, M., Harata, M. *Biomacromolec.* 2002, *3*, 1-4.

3. SUMMARY OF THE INVENTION

The invention encompasses methods of depositing a thin layer of the polysaccharide chitosan onto the surface of an electrode substrate. The methods comprise contacting the substrate with a chitosan solution and applying an electric current to the substrate. The invention also encompasses substrates onto which a layer of chitosan has been deposited.

3.1 FIGURES

Various aspects of the invention may be understood with reference to the following figures:

FIG. 1 represents a diagram of chitosan deposition;

FIG. 2 provides a graphical representation of the deposition of chitosan onto the surface of an electrode, wherein deposition occurred from a 1 w/v% chitosan solution using an applied voltage of 2.5 V;

FIG. 3 provides an SEM micrograph of a deposited layer on an electrode (a) without neutralization and (b) with neutralization;

FIG. 4 represents deposition under the following conditions, each of which include immersing the electrode in caustic, rinsing it extensively and drying it prior to measuring the thickness: (a) deposition occurring from a 1 w/v% chitosan solution using an applied voltage of 2.5 V; (b) deposition measured after 6 minutes using chitosan solutions of varying concentrations and an applied voltage of 2.5 V; (c) deposition measured after 6 minutes from a 1 w/v% chitosan solution using varying voltages;

FIG. 5 provides an IR spectrum of deposited material and chitosan, wherein the material deposited on the electrode was neutralized in base, extensively washed with distilled water, and dried; the IR spectrum was collected using a KBr pellet; and the control spectrum was collected using a chitosan film; and

FIG. 6 provides an ES-MS spectrum of deposited material after incubation with chitosanase.

4. DETAILED DESCRIPTION OF THE INVENTION

As used herein and unless otherwise indicated, a "substrate" is a material upon which chitosan can be deposited. Suitable substrates are electrically conducting, and are made of materials such as, but not limited to, metals (*e.g.*, aluminum, antimony, cadmium, chromium, cobalt, copper, gold, iron, lead, magnesium, mercury, nickel, palladium, platinum, silver, steel, tin, tungsten, zinc, and alloys thereof) semiconductors, and conductive polymers.

As used herein and unless otherwise indicated, a "cell" may be eucaryotic or prokaryotic and may be from any source where cells can be obtained.

For the chitosan solution used to deposit chitosan onto a substrate, suitable concentrations of chitosan vary from about 0.0001 to about 0.001 (w/v) %, about 0.001 to about 0.01 (w/v) %, about 0.01 to about 0.1 (w/v) %, about 0.1 to about 1 (w/v) %, about 1 to about 10 (w/v) %, about 10 to about 20 (w/v), and about 20 to about 30 (w/v) %. A suitable pH for deposition of chitosan onto a substrate is any pH where chitosan remains soluble and in solution. It is further recognized that the concentration of the chitosan solution, the voltage and the time a current is applied to deposit chitosan onto a substrate can be varied to control the extent of chitosan deposition.

In a specific embodiment of the present invention, the method of depositing chitosan onto a metal substrate comprises: a) contacting the substrate with a solution containing chitosan; and b) applying an electric current to the substrate, sufficient to deposit chitosan onto the substrate. In another specific embodiment, the method of depositing chitosan onto a metal substrate further comprises washing the substrate containing deposited chitosan with at least one liquid selected from the group consisting of water, a solution with neutral pH, a basic solution and an acidic solution. In another specific embodiment, the method of depositing chitosan onto a metal substrate further comprises contacting the chitosan-bound substrate with chitosanase.

A specific embodiment of the present invention is a substrate coated with chitosan. In a particular embodiment, the thickness of the chitosan is from about 0.01 to about 3 microns, from about 0.01 to about 1.5 microns, or from about 0.02 to about 0.8 microns.

A further specific embodiment is a substrate coated with chitosan further comprising bound protein. Another specific embodiment is a substrate coated with chitosan further comprising a bound enzyme. Another specific embodiment is a substrate coated with

chitosan further comprising bound polynucleotide. Yet another specific embodiment is a substrate coated with chitosan further comprising either bound RNA or DNA. Still another specific embodiment is a substrate coated with chitosan further comprising bound cells. A further specific embodiment of the inventions is a substrate coated with chitosan wherein the substrate is a metal.

5. Example

Chitosan from crab shells (85 % deacetylation as reported by the supplier) and the enzyme chitosanase were purchased from Sigma-Aldrich Chemicals. Chitosanase was reported by the manufacturer to have specific activities of 102.3 U/mg. Chitosan solutions were prepared by adding chitosan flakes to water and incrementally adding small amounts of HCl to the solution to maintain the pH near 3. After filtering undissolved material, these chitosan solutions were diluted to various concentrations, and the pH was adjusted to 5.0 using NaOH (1 M).

Electrodes were prepared by depositing 90 Å thick chromium (Cr) and then 2000 Å thick gold (Au) films on 4-inch diameter silicon wafers already coated with 1-micron thick thermal oxide film. For chitosan deposition, the electrodes were dipped into a chitosan solution (pH=5, 1% w/v) as shown in FIG. 1. In most experiments, three electrodes were examined. Two of the electrodes (positive and negative) were connected to a DC voltage supply using alligator clips. The third electrode was not connected to a power supply and is designated a "neutral" electrode. At specific times the electrodes were removed from the solution and rinsed with distilled water, after which the voltage was removed. In some cases, electrodes were immediately oven-dried (60 °C for 3 hours). In other cases, electrodes were neutralized by immersion in a basic solution (1 M NaOH) and then rinsed with distilled water prior to drying. After drying, the thickness of the deposited layers was measured by a profilometer (ALPHA-STEP 500 SURFACE PROFILER, TENCOR Instruments). Thicknesses were measured on different areas of the electrode surface and the average values were calculated.

Scanning electron microscopy (SEM) was used to study the surface morphology of the deposited layer. SEM micrographs have been recorded using a Focused Ion Beam system (FIB/SEM workstation dual beam 620; FEI Company). Samples on silicon substrates were

placed in the chamber having vacuum of about 10^{-6} Torr. Structural properties were examined at a 20,000-fold magnification.

For chemical analysis, deposition was obtained by placing electrodes in a chitosan bath (1 w/v %; pH = 5) for 20 minutes with an applied voltage of 2.0 volts. For IR analysis, the negative electrode was removed from the chitosan solution, rinsed, disconnected from the power supply, and then placed in about 1 M NaOH overnight. When the electrode was soaked in base for such a long time, the deposited material was observed to detach from the electrode surface. This deposited material was then extensively washed with distilled water and dried overnight at 60 °C. After drying, it was ground with KBr powder and pressed into a pellet. IR spectra were collected using a Perkin-Elmer 2000 FT-IR system.

For analysis by electrospray mass spectrometry (ES-MS), the negative electrode was removed from the chitosan solution, rinsed, disconnected from the power supply, and then placed in a small volume of dilute acid (HCl; pH=3) and held overnight to allow the deposited material to dissolve. This acid solution was recovered, diluted to approximately 0.08 w/v % and the pH was adjusted to 5.5. The sample was then incubated for one day at 37°C with the enzyme chitosanase (0.2 U/ml). After incubation the solution was filtered to remove precipitates, and analyzed by ES-MS (Thermo Finnigan, San Jose, CA, USA). All samples for ES-MS analysis were diluted in an aqueous solution containing 0.1 % formic acid and analyzed in positive ion mode.

To examine deposition, we immersed electrodes in an acidic chitosan solution and applied a voltage of 2.5 V. After applying the voltage for varying times, negative electrodes were removed from the solution, rinsed with distilled water, and the voltage was removed. In some cases, the electrodes were dried, while in other cases they were immersed in base, rinsed and then dried. After drying, the thickness of the deposited layer was measured by profilometry. Figure 2 shows that the thickness of the deposited layer increases over time. Additionally, Figure 2 shows that the thickness of the deposited layer is less when the electrode was treated with base.

Scanning electron microscopy (SEM) was used to examine the surface morphology of the negative electrodes. Figure 3a shows micrographs for electrodes that were dried without neutralization. As can be seen from Figure 3a, this sample has a non-uniform surface morphology. Possibly, the surface roughness of this electrode may be due to the presence of

salts associated with the chitosan polyelectrolyte. Figure 3b shows the surface of a negative electrode that had been immersed in base and rinsed extensively before drying. As indicated in Figure 3b, the surface of this electrode is more uniform – presumably due to the neutralization of chitosan. The observation in Figure 2 that deposited layers are thinner after neutralization suggests that neutralization leads to a collapse of the polymer network and possibly also the elimination of salts. In subsequent experiments, neutralization was performed prior to measuring the thickness of deposited layers.

Additional studies were performed to characterize deposition, and to compare deposition onto the negative and positive electrodes. Figure 4a shows that the thickness of the deposited layer on the negative electrode increased over time. No material was observed to deposit on the positive electrode under the conditions studied. An additional control was an electrode in which no voltage was applied (designated as "neutral" electrode). As shown in Figure 4a, no deposition was observed on the surface of this "neutral" electrode. Figure 4b shows that when the concentration of chitosan in the solution was increased, there was increased deposition on the surface of the negative electrode. Again no deposition was observed on the positive electrode or on the control electrode in which no voltage was applied. Figure 4c shows that deposition on the negative electrode also increased with increasing voltage.

In summary, Figures 2 through 4 demonstrate that an applied voltage can be used to deposit a thin layer onto a negative electrode when the electrode is immersed in a chitosan solution. Additionally, the thickness of the deposited layer can be controlled by the deposition conditions. Finally, once the deposited layer is neutralized, it can be retained on the electrode surface even in the absence of an applied voltage (*i.e.*, the electrode can be extensively rinsed). This latter observation is consistent with the fact that chitosan is insoluble under neutral and basic conditions.

Two independent techniques were used to provide chemical evidence that the material deposited on the negative electrode is chitosan. For IR analysis, the "neutralized" material was recovered from the electrode surface, rinsed extensively, dried overnight, and incorporated into a KBr pellet. Figure 5 compares the IR spectrum for the KBr pellet of the deposited material with the spectrum of a chitosan film. The absorption spectra are similar for the two samples providing evidence that the material deposited on the negative electrode

is chitosan. Some differences in the spectra are observed in the amine and amide regions (1500-1700 cm^{-1}) suggesting the possibility that chitosan chains that are more highly deacetylated (and therefore more highly charged) may be preferentially deposited onto the negative electrode. Sannan, T., Kurita, K., Ogura, K., Iwakura, Y. *Polymer* **1978**, *19*, 458-459; Domszey, J. G., Roberts, G. A. F. *Makromol Chem.* **1985**, *186*, 1671-1677; Shigemasa, Y., Matsuura, H., Sashiwa, H., Saimoto, H. *Int. J. Biol. Macromol.* **1996**, *18*, 237-242.

The second technique to provide chemical evidence that the deposited material is chitosan was provided by electrospray mass spectrometry (ES-MS). Because chitosan's molecular weight (> 300,000 g/mol) exceeds the limit for analysis, we enzymatically hydrolyzed the deposited material and analyzed the hydrolysate. For this analysis, the deposited layer was dissolved from the electrode surface into an acidic solution. After dilution, the solution was incubated with the chitosan-hydrolyzing enzyme, chitosanase. Osswald, W.F., McDonald, R.E., Nied, R.P., Shapiro, J.P., Mayer, R.T. *Anal. Biochem.* **1992**, *204*, 40-46. Figure 6 shows the ES-MS results for this hydrolyzate.

To examine the results in Figure 6, we considered the peaks expected for the enzymatic hydrolysis of chitosan. Enzymatic hydrolysis of chitosan is known to result in the formation of various species (*e.g.*, monomers, dimers). Shahgholi, M., Callahan, J. H., Rappoli, B. J., Rowley, D. A. *J. Mass Spectrom.* **1997**, *32*, 1080-1093. Additionally, chitosan is a copolymer of glucosamine and N-acetylglucosamine, and the predominant oligomeric species are expected to consist of either glucosamine units or both glucosamine and N-acetylglucosamine units. Because the degree of acetylation is low (15 %), it is not expected that significant amounts of oligomers that contain more than a single N-acetylglucosamine residue. Finally, it is known that MS spectra of glucosamine and glucosamine trimers contain product ions resulting from the loss of H_2O. Kerwin, J. L., Whitney, D. L., Sheikh, A. *Insect Biochem. Molec.* **1999**, *29*, 599-607. Table 1 lists a series of peaks expected for the hydrolysis of chitosan (*e.g.*, various monomers, dimers, trimers, tetramers, and pentamers). By comparison of these expectations with results in Figure 6 (listed in parenthesis in Table 1), it is clear that the ES-MS provides strong evidence that the deposited material is chitosan.

A control in the ES-MS study was provided by a sample that was incubated in the absence of chitosanase. The ES-MS analysis of this control showed weak signals with a low signal-to-noise ratio (not shown). This is consistent with the expectation that un-hydrolyzed

chitosan will be too large (300,000 g/mol) to be measured by ES-MS. The highest signals in this control appeared at *m/z* of 220 and 299 and the latter signal does not even appear in Figure 6. Thus, without being limited by theory, chitosanase-catalyzed hydrolysis of the deposited material may be necessary to attain strong signals in the ES-MS.

Table 1. Expected and observed *m/z* values for enzymatically hydrolyzed chitosan. (Observed values from Figure 6 appear in parenthesis)

	Monomer	Dimer	Trimer	Tetramer	Pentamer
$(Gln)_x - 3H_2O$	126 (126)	287 (288)	448 (448)	609 (609)	770 (769)
$(Gln)_x - 2H_2O$	144 (144)	305 (306)	466 (467)	627 (625)	788 (789)
$(Gln)_x - H_2O$	162 (162)	323 (324)	484 (484)	645 (644)	806 (805)
$(Gln)_x$	180 (180)	341 (342)	502 (503)	663 (663)	824 (821)
$[GlcNAc \bullet (Gln)_{x-1}] - H_2O$	204 (205)	365 (364)	526 (525)	687 (686)	848 (847)
$[GlcNAc \bullet (Gln)_{x-1}]$	222	383	544 (545)	705 (705)	866 (864)

Gln: Glucosamine; GlcNAc: N-Acetylglucosamine.

In summary, two independent techniques were used to provide chemical evidence that the deposited material was chitosan. Standard IR analysis shows that the spectrum for the deposited material is similar to the spectrum for chitosan. Further, the deposited material was susceptible to hydrolysis by the enzyme chitosanase while the hydrolysate shows a family of peaks consistent with glucosamine and N-acetylglucosamine residues – the repeating units of chitosan.

Chitosan is a unique biopolymer that offers interesting possibilities for controlling the surface chemistry of devices. First, chitosan is an amine-rich polysaccharide that is

positively charged under mildly acidic conditions. This characteristic allows a thin chitosan layer to be deposited (*i.e.*, "self-assembled") onto a negative electrode in response to an applied voltage. The results reported here demonstrate that the thickness of the deposited layer can be controlled by the conditions used. Second, chitosan's pKa is rather low (pKa ≈ 6.3) compared to other amine-containing biopolymers (*e.g.*, polylysine's pKa is 10.5), and above it's pKa chitosan is insoluble. As a result of this pH-dependent solubility, a simple neutralization step is sufficient to convert chitosan to an insoluble form that can be retained on the surface of the electrode (*i.e.*, the applied voltage is only required for deposition and not to retain the chitosan layer). Third, the high content of primary amine groups allows a chitosan coating to be used for controlling surface properties and for subsequent modification steps. The utility of amine groups is illustrated by the current interest in creating amine-terminated monolayers. Whitesides, G. M., Laibinis, P. E. *Langm.* **1990**, *6*, 87-96; Gole, A., Sainkar, S.R., Sastry, M. *Chem. Mater.* **2000**, *12*, 1234-1239; Sieval, A.B., Linke, R., Heij, G., Meijer, G., Zuilhof, H., Sudholter, E.J.R. *Langm.* **2001**, *17*, 7554-7559; Wallwork, M.L., Smith, D.A., Zhang, J., Kirkham, J., Robinson, C. *Langm.* **2001**, *17*, 1126-1131; Nishiyama, K., Kubo, A., Ueda, A., Taniguchi, I. *Chem. Lett.* **2002**, (1), 80-81; Jiang, X., Ortiz, C., Hammond, P.T. *Langm.* **2002**, *18*, 1131-1143. The amine groups also enable biologically active molecules (*e.g.*, peptides and proteins) to be coupled onto chitosan surfaces using standard coupling chemistries (*e.g.*, glutaraldehyde- or carbodiimide- based chemistries) or using enzymatic methods. Vazquez-Duhalt, R., Tinoco, R., D'Antonio, P., Topoleski, L.D.T., Payne G.F. *Bioconj. Chem.*, **2001**, *12*, 301-306. Finally, chitosan is gaining increasing attention as a biomaterial for applications ranging from enzyme immobilization to the creation of biocompatible surfaces. Airoldi, C., Monteiro, O.A.C. *J. Appl. Polym. Sci.* **2000**, *77*, 797-804; Belmonte, M.M., De Benedittis, A., Muzzarelli, R.A.A., Mengucci, P., Biagini, G., Gandolfi, M.G., Zucchini, C., Krajewski, A., Ravaglioli, A., Roncari, E., Fini, M., Giardino, R. *J. Mater. Sci.-Mater. Med.* **1998**, *9*, 485-492; Lvov, Y., Onda, M., Ariga, K., Kunitake, T. *J. Biomat. Sci. – Polym. Ed.*, **1998**, *9*, 345-355; Wang, D.A., Ji, J., Sun, Y.H., Yu, G.H., Feng, L.X. *J. Biomed. Mater. Res.* **2001**, *58*, 372-383; Gong, H. P., Zhong Y. H., Li, J. C., Gong, Y. D., Zhao, N. M., Zhang, X.F. *J. Biomed. Mater. Res.* **2000**, *52*, 285-295. Thus, chitosan may provide an appropriate interface between biological systems and microelectronic devices.

The prior example is provided as illustration of the disclosed invention and is not intended to limit the scope of the invention. All cited references are herein incorporated in their entireties by reference.

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CLAIMS

We claim:

1. A method of depositing chitosan onto a substrate, comprising:

a) contacting the substrate with a solution containing chitosan; and

b) applying an electric current to the substrate sufficient to deposit the chitosan onto the substrate.

2. The method of claim 1, further comprising washing the substrate containing deposited chitosan with water, a solution with a neutral pH, a basic solution, or an acidic solution.

3. The method of claim 1, further comprising contacting chitosan deposited on the substrate with chitosanase.

4. The method of claim 1, wherein the substrate is a semiconductor.

5. The method of claim 1, wherein the substrate is a conductive polymer.

6. The method of claim 1, wherein the substrate is a metal.

7. The method of claim 1, wherein the solution contains chitosan in a concentration of from about 0.0001 to about 30 % w/v.

8. The method of claim 7, wherein the solution contains chitosan in a concentration of from about 0.1 to about 10 % w/v.

9. A material obtained by the method of claim 1.

10. A material comprising chitosan deposited on a substrate.

11. The material of claim 10, wherein the substrate is a metal, a semi-conductor, or a conductive polymer.

12. The material of claim 11, wherein the substrate is a metal.

13. The material of claim 12, wherein the metal is aluminum, antimony, cadmium, chromium, cobalt, copper, gold, iron, lead, magnesium, mercury, nickel, palladium, platinum, silver, steel, tin, tungsten, zinc, or an alloy thereof.

14. The material of claim 10, further comprising a protein bound to the chitosan.

15. The material of claim 10, further comprising an enzyme bound to the chitosan.

16. The material of claim 10, further comprising a polynucleotide bound to the chitosan.

17. The material of claim 16, wherein the bound polynucleotide is RNA.

18. The material of claim 16, wherein the bound polynucleotide is DNA.

19. The substrate of claim 10, further comprising cells bound to the chitosan.

ABSTRACT

The deposition of chitosan onto electrode surfaces is disclosed. Methods of depositing chitosan on surfaces are disclosed. Materials comprising chitosan deposited on a substrate are also disclosed.

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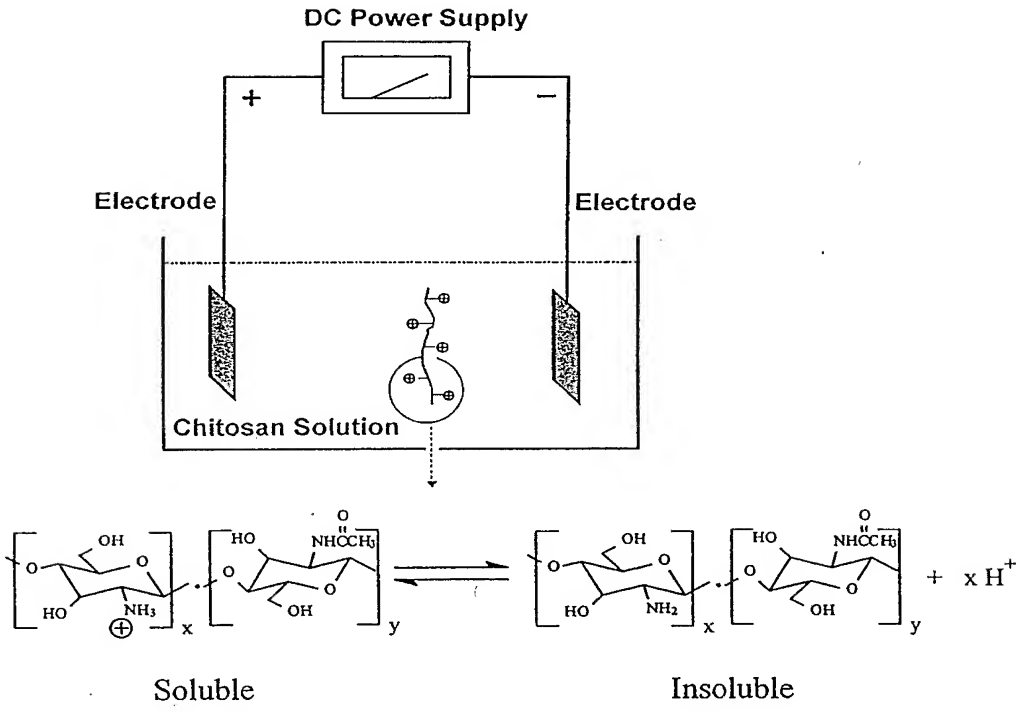

DC Power Supply
+
−
Electrode
Electrode
Chitosan Solution
Soluble
Insoluble
+ x H+

FIG 1

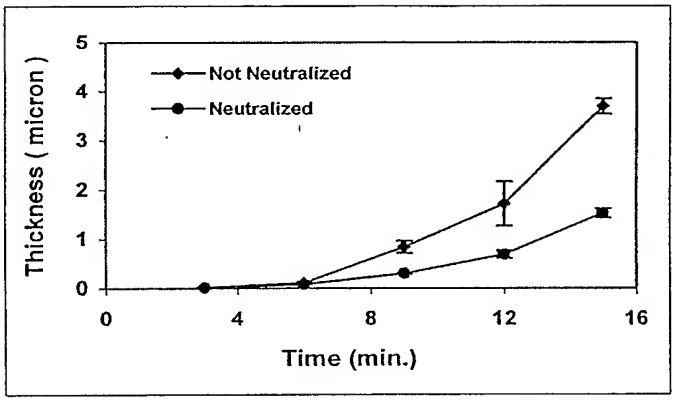
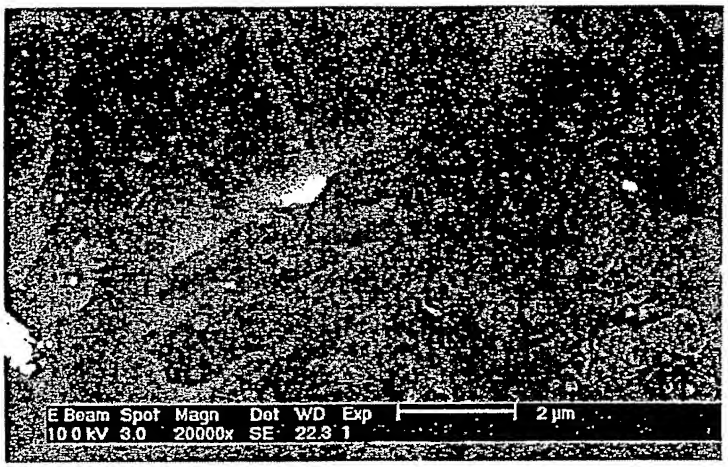
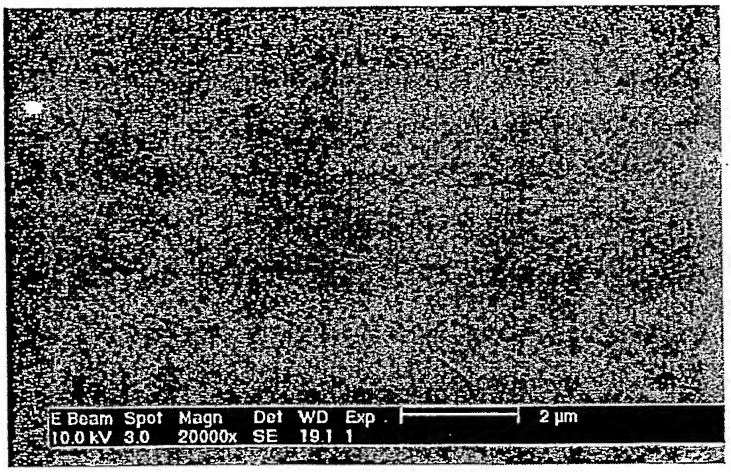

Thickness (micron)
5
4
3
2
1
0
Not Neutralized
Neutralized
0
4
8
12
16
Time (min.)

FIG. 2

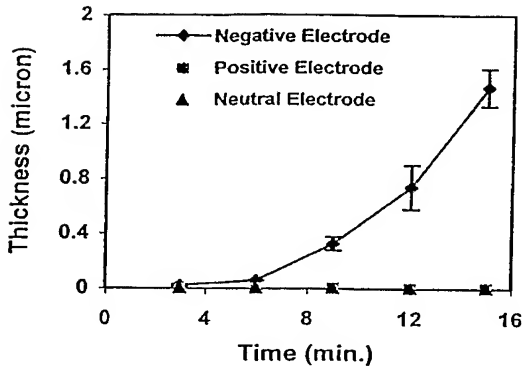

E Beam Spot Magn Det WD Exp
10.0 kV 3.0 20000x SE 22.3 1
2 µm

(a)

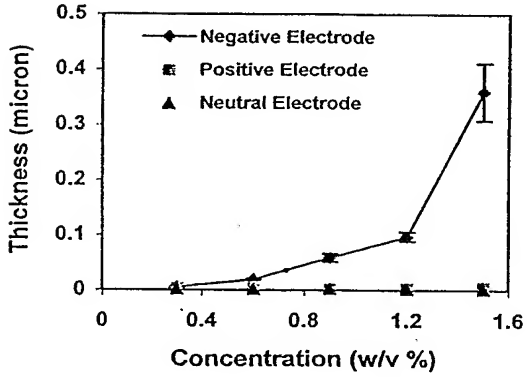

E Beam Spot Magn Det WD Exp
10.0 kV 3.0 20000x SE 19.1 1
2 µm

(b)

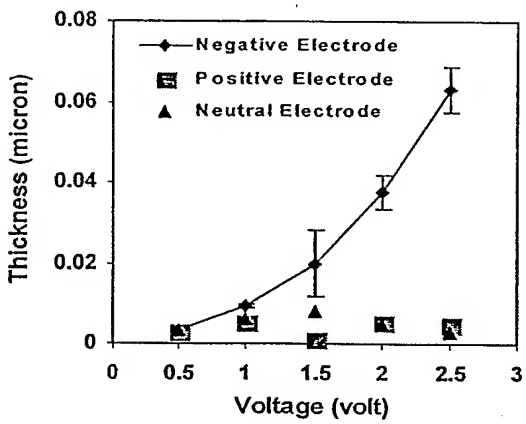
FIG 3


Negative Electrode
Positive Electrode
Neutral Electrode
Thickness (micron)
Time (min.)
0
0.4
0.8
1.2
1.6
2
4
8
12
16

(a)


Negative Electrode
Positive Electrode
Neutral Electrode
Thickness (micron)
Concentration (w/v %)
0
0.1
0.2
0.3
0.4
0.5
0.4
0.8
1.2
1.6

(b)


Negative Electrode
Positive Electrode
Neutral Electrode
Thickness (micron)
Voltage (volt)
0
0.02
0.04
0.06
0.08
0.5
1
1.5
2
2.5
3

(c)

FIG. 4

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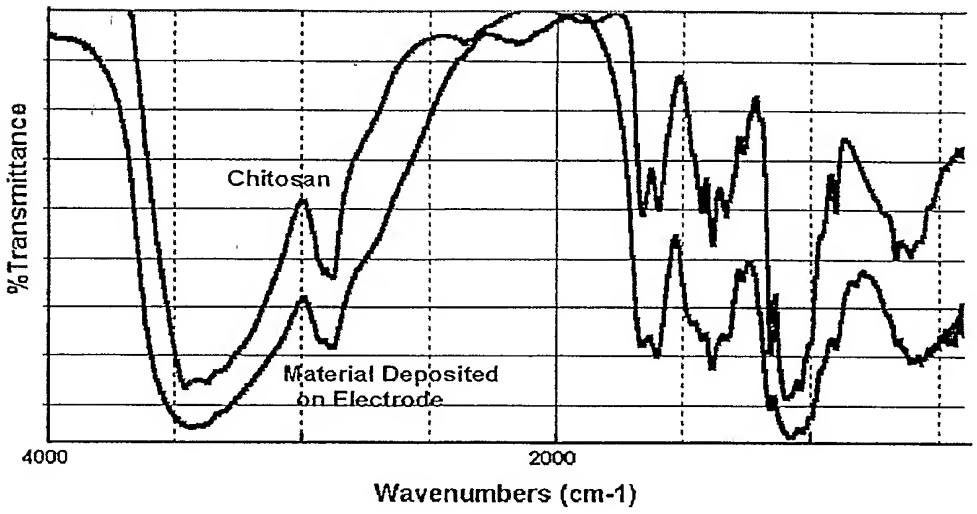
%Transmittance
Chitosan
Material Deposited
on Electrode
4000
2000
Wavenumbers (cm-1)

FIG. 5

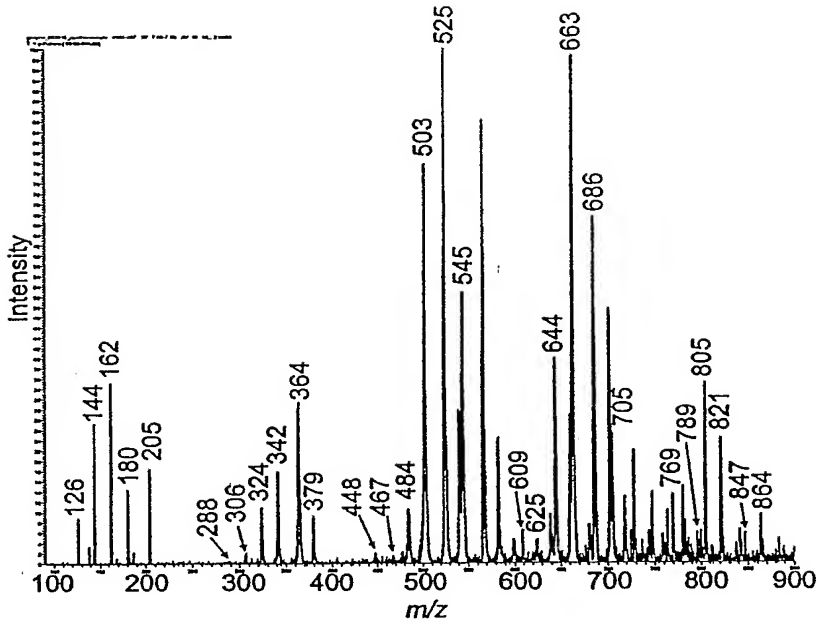
Intensity
126
144
162
180
205
288
306
324
342
364
379
448
467
484
503
525
545
609
625
644
663
686
705
769
789
805
821
847
864
100
200
300
400
500
600
700
800
900
m/z

FIG. 6